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Uptake of Trace Metals by the Clam *Macoma inquinata* from Clean and Oil-Contaminated Detritus

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In recent years there has been increasing concern about the entry of petroleum hydrocarbon (PHC) into the marine environment and the effects of such entry on the composition and functioning of the marine ecosystem. Few reports have been published on the possible effect of oil on the uptake of metals from water or sediments by animals. The possibility of such effects is indicated by the work of FLETCHER et al. (1979), who showed that crude oil causes a reduction in blood plasma copper concentrations in fish, and PAYNE et al. (1978) who reported that petroleum affected chloride regulation in fish. LUOMA and JENNE (1977) have shown that the availability of sediment-bound metals to a deposit-feeding clam depended on the metal-sediment association and sediment-to-water desorption rate.

Here, we exposed a detritivorous clam, Macoma inquinata, to clean and oil-contaminated detritus to determine the effects of the oil on metal accumulation. To measure the uptake of metals, clams were exposed to neutron activated detritus and the uptake of several isotopes (^{51}Cr, ^{60}Co, ^{152}Eu, ^{59}Fe, ^{46}Sc, and ^{65}Zn) measured in the clams.

MATERIALS AND METHODS

Preparation of detritus

The term "detritus" as used in this paper is defined as the suspended matter in Sequim Bay water that settled out in the head tanks of the laboratory flowing seawater system. The element composition of this detritus is similar to both that of Sequim Bay fine grained sediment and shale (Vinogradov 1962).

Detritus was collected from the bottom of head tanks through which raw Sequim Bay seawater had passed. Dried detritus was neutron activated for two hours at a neutron flux of approximately 1×10^{13} n cm $^{-2}$ sec $^{-1}$, and stored for three months to allow activity to be reduced through radioactive decay. Six g dry weight (d.w.) of neutron activated detritus was added to 100 g wet weight of untreated detritus in 1 L of sea water, and the mixture was shaken by hand for 1 minute. The resulting slurry was aerated for five days at 13°C, then filtered onto #42 Whatman paper and the detritus divided into two portions of approximately 60g each. Enough Prudhoe Bay crude oil to produce a concentration of 1000 ppm oil in 60g of the detritus was dissolved in 1 ml of ether. The ether-oil mixture was then added to 100 ml sea water

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containing 60g of detritus. To the second 60 g portion of detritus was added 100 ml seawater and 1 ml ether without oil. Each portion of detritus was shaken 4 minutes, filtered again, and samples of the detritus removed for gamma counting.

Exposure system

The exposure system was designed to expose clams to either oiled or non-oiled detritus, and also expose other clams to only water-borne materials defined as less than 100 μm in diameter. The exposure system consisted of two 4 L aquaria that had been divided into two equal compartments. Oiled or non-oiled detritus was placed in one compartment of each aquaria and 2 L seawater added to each compartment. Water was pumped from the compartment containing detritus through a 100 μm mesh nylon screen into the adjacent compartment. Water returned to the detritus containing compartment by gravity flow over a separating barrier. The clams were dug and stored in a flowing aquarium without food, for two days before the experiment began. Thirty clams were placed in each of the four compartments. To maintain a constant temperature the aquaria were placed in a 13° water bath.

This system provided for the exposure of clams to detritus and water-borne materials (<100μm) on one side and only water-borne materials (<100μm) on the other. The pump system provided the necessary aeration without agitating the detritus enough to suspend it in the water column. There was a small amount of solids that accumulated on the bottom in the filtered compartment. These were removed periodically by gentle suction and returned to the compartments with detritus.

Groups of five clams were removed from each compartment after 2, 4, 8, and 15 days of exposure. They were opened, and the meat and shells were rinsed in fresh sea water. If needed, shells were scrubbed before being opened to remove adhering detritus. The meat and shells of each group were dried to constant weight at 80°C. One-hundred ml samples of sea water were taken from the filtered compartment after 2, 8, and 15 days and evaporated to dryness for counting. Samples of oiled and non-oiled detritus were removed and dried on the 15th day of exposure, for gamma counting.

After 15 days of exposure, the remaining clams were transferred to depuration tanks with clean water and non-labelled detritus, both of which were replaced after 2 and 4 days. Groups of five clams were removed after 2 and 8 days of depuration and prepared for counting. All meat and shells from 1 group were pooled. The gamma activity of samples of detritus, meat, shells, and residue from evaporated sea water was measured on a Ge(Li) diode.

Data treatment

The numbers of net counts at energy levels corresponding to ^{51}Cr, ^{152}Eu, ^{46}Sc, ^{59}Fe, ^{65}Zn, and ^{60}Co were calculated and corrected for the rates of decay of each isotope to determine the count rate/g d.w./1000 minutes at the time each sample was removed from the experiment. The relation between the corrected isotope count and the actual amount of metal present was established by

reference to the known metal content of the detritus. These values had been established in this laboratory for K, Ca, Ti, V, Cr, Mn, Fe, Cu, Zn, Se, Pb, and As, by x-ray fluorescence. The elemental values we measured agreed with the trace metal concentrations in shale as reported by KRAUSKOPF (1967); taken from VINOGRADOV (1962). Since the elemental composition of the collected detritus was similar to that of shale, we used the levels of Eu, Sc, and Co in shale in calculations to determine the amount of metal originating from detritus that was present per g clam meat or shell, or per ml sea water.

Since the clams that were removed from the aquaria during the exposure had not been depurated to purge their intestinal tracts before they were shucked and dried, it was necessary to partition the total isotope content of each metal in meat samples into that portion which was in transit through the animals' digestive systems at the time of sampling and that which had passed through the gut wall and been incorporated into the tissues. This partitioning was made possible by the presence of ^{46}Sc and ^{152}Eu in the detritus. Based on work by PALUMBO (1963) and PETERS and HOSS (1974) there is good reason to believe that these two elements, as a result of their chemical form and insolubility, are absorbed very poorly if at all from food into animal tissue. The amount of detritus localized in the gut lumen was, therefore, taken to be equal to the amount of shale which would contain the scandium present in the entire sample. The amounts of other metals localized in the gut were calculated by multiplying the concentration of each metal in shale by the calculated quantity of detritus in the gut. To determine the amount of each labelled metal in tissue, the amount present in the gut was subtracted from the total amount present in the sample.

One example of this treatment of the data is given here:

Metal	Concen. in shale	Concen. in detritus	Corrected Counts /1000 mins/g d.w. detritus	Corrected Counts /1000 mins/ g d.w. Macoma (non-oiled, 4 days)
Sc	10µg/g		287,907	4086
Zn	80µg/g	88µg/g	3,493	90

$\frac{3493}{88} = \frac{90}{x}$ $\quad x = 2.27$ µg Zn/g d.w. total Macoma sample.

$\frac{287,907}{10} = \frac{4086}{y}$ $\quad y = .142$ µg Sc/g d.w. total Macoma sample.

$\frac{10}{10^6} = \frac{.142}{z}$ $\quad z = 14,200$ µg = 14.2 mg detritus/g d.w. total Macoma sample.

$\frac{14.2 \times 88}{1000} = 1.25$ µg Zn/g d.w. sample, associated with detritus.

$2.27 - 1.25 = 1.02$ µg Zn/g d.w. sample, incorporated into tissue.

RESULTS

The only metal which appeared consistently at detectable levels in sea water was cobalt, whereas Cr, Eu, and Sc appeared sporadically and no Fe or Zn were detected. As shown in Table 1, the amount of Co released to the sea water by oiled and non-oiled sediment did not differ.

TABLE 1

Co content of filtered sea water ($\mu g \times 10^{-5}/ml$).

Days Exposure	Oiled	Non-oiled
2	5.1	4.5
8	4.15	no sample
15	6.0	5.5

The detrital contents, as calculated from the scandium levels of the samples, indicated that the clams fed during the first two days of direct exposure to detritus, and the net amount of food in their digestive tracts declined thereafter as shown in Table 2 and Figure 1. On day 2, in the absence of oil, 25.6 mg/g of body d.w. was composed of detritus. In the presence of oil only one half as much food was ingested initially and it was lost at a greater rate than in the non-oiled clams.

In the filtered compartment of the aquarium to which no oil had been added, less than one tenth as much scandium labelled material, assumed to be detritus, was taken up initially as in the non-oiled detritus compartment. The Sc level in clams in the filtered compartment declined more slowly and irregularly than that in clams in the detritus compartment. Clams receiving filtered water from oiled detritus took in about as much food as the non-oiled controls.

Following the transfer of clams to tanks containing non-labelled detritus, part of the radioactive material was quickly lost from the gut, but a fraction, on the order of one to ten percent of the originally ingested material, remained at the end of eight days.

TABLE 2

Calculated weight of detritus (mg/g d.w.) in *Macoma inquinata*.

Days Exposure	Non-oiled	Oiled	Non-oiled (filtered)	Oiled (filtered)
2	25.7	13.5	1.53	1.99
4	14.2	8.31	0.96	0.57
8	10	2.97	1.52	0.37
15	6.2	1.07	0.88	0.38
Days Depuration				
2	1.1	0.88	0.22	0.14
8	0.37	0.24	0.15	0.14

Table 3 shows the amounts of Zn and Co per g d.w. incorporated into Macoma tissue. Chromium was detected in only three clam samples. The levels of Eu present in the total samples differed from the amounts calculated to be in the detritus by less than 10^{-2} µg/g in all but one case. Probably, these small differences were artifacts produced by the random nature of the gamma-emitting process, and europium, like other rare earths, is not absorbed from the gut. Similarly, the apparent net uptake of iron was so irregular and represented such a small proportion of the iron present in the detritus that it does not provide firm evidence that any iron was taken into the tissue.

Figures 2 and 3 show the amounts of Co and Zn incorporated into Macoma tissues during two weeks exposure to labelled detritus and one week depuration. Those organisms which received their metal through the water column or on very fine particles incorporated nearly identical amounts whether oil was present or absent. Those which had direct access to detritus incorporated the same amounts early in the exposure period, but later the oiled animals took in less. This difference is probably due to the fact that less oiled than non-oiled detritus was ingested and, therefore, a smaller amount of labelled metal was available for absorption across the walls of the intestinal tract.

The only metal to appear consistently in the shells was cobalt. Its concentration there was of the same order of magnitude as in the clam meat. There was no indication that more cobalt was taken into the shells from oiled than from non-oiled detritus.

DISCUSSION

The detritus on which the clams fed in this experiment was approximately the same material they ingest in nature. There is, therefore, no reason to believe that the concentrations of metals in their food were any higher during than before the experiment or to expect a net increase in metal concentration in the controls, i.e., those animals exposed to non-oiled detritus. The fact that labelled Zn and Co appeared in the controls indicates that a more or less rapid exchange takes place between the metals in the tissue and those in the food or water. The persistence of some labelled metals during the period of depuration may be caused by the retention of old material in the animals' gut after their transfer to clean aquaria.

The normal tissue zinc concentration of Macoma is about 200 ppm. The cobalt concentration is not known, but probably resembles that of other bivalves from unpolluted waters, which is on the order of 0.5 ppm. Thus, the amount of labelled zinc and cobalt taken in by non-oiled Macoma in two weeks, presumably replacing metals lost to the environment, amounts to about 1% and 30%, respectively, of their normal metal pool. If hydrocarbons enhanced the rate of uptake of metal from detritus, then oil-exposed animals would be expected to accumulate metals from the detritus at a faster rate than that at which they lost them to the environment and so to exhibit a greater increase in radioactivity than is found in the non-oil exposed clams. This was not the case, since the net amount of radioactivity incorporated into the tissues of oil-exposed clams over time was less than that in the

TABLE 3

Zn and CO (µg/g d.w. tissue) incorporation into Macoma inquinata exposed to oiled and non-oiled detritus.

	Zn				Co			
Days Exposure	NO	O	NOF	OF	NO	O	NOF	OF
2	0.24	0.29	0.26	-	0.024	0.059	0.009	0.019 (0.009)
4	1.02	1.38	0.32	-	0.070 (0.038)	0.112	0.025	0.043
8	1.18	1.09	0.572	0.95	0.175 (0.038)	0.108 (0.033)	0.049 (0.088)	0.043 (0.022)
15	3.41	1.38	1.05	0.87	0.140 (0.051)	0.067	0.042	0.054
Days Depuration								
2	2.23	0.62	0.94	1.01	0.216	0.130	0.043 (0.066)	0.026 (0.026)
8	2.10	0.99	1.10	1.32	0.133	0.080	0.037 (0.038)	0.046

O = oiled
NO = not oiled
F = filtered
- = no metal present in sample
figures in () = concentrations in shells

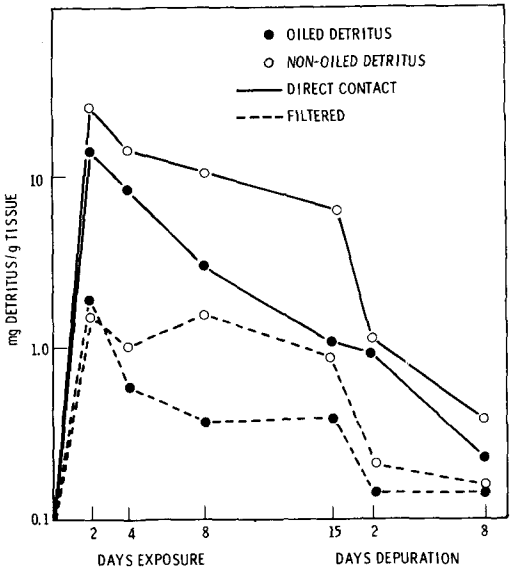

Figure 1. Detritus ingested by Macoma inquinata.

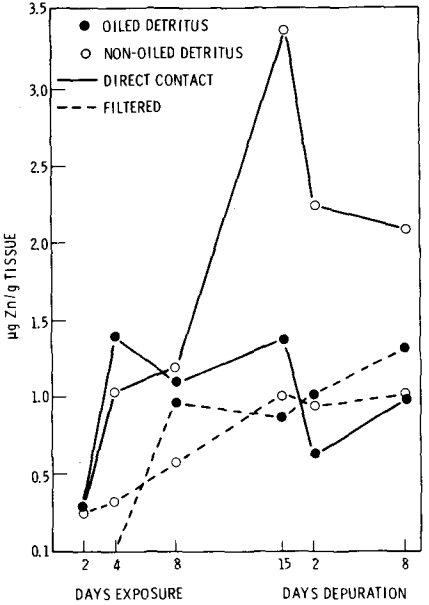

Figure 2. Incorporation of radiolabelled zinc into Macoma tissue.

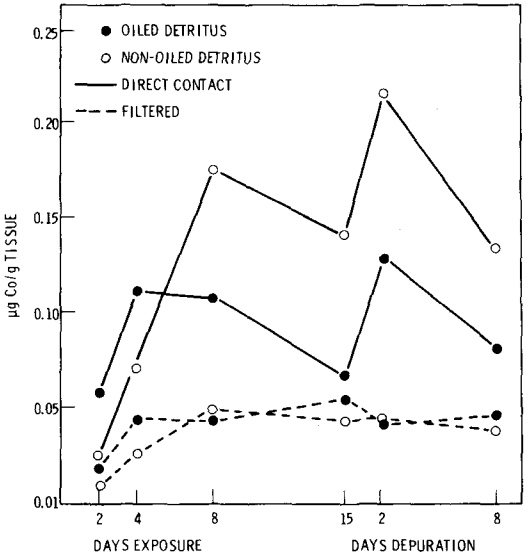

Figure 3. Incorporation of radiolabelled cobalt into Macoma tissue.

tissues of clams exposed to non-oiled detritus. This reduction in metal uptake by tissue, however, does not imply that PHCs reduce the ability of Macoma to absorb metals from detritus, but that PHC reduces the feeding rate of exposed clams.

There seem to be no grounds for believing that exposure to 1000 ppm PHC either increases or decreases the rate at which Macoma absorbs metals, except through a reduction in the rate of food intake. This conclusion is supported by the fact that in the filtered compartment of the aquarium, where the absolute differences between food intakes of oiled and non-oiled animals were less and where more of the metals were probably taken in via the water column, the amounts incorporated by the two groups were quite similar. The results of this experiment therefore indicate that, while the presence of crude oil in sediment may affect the clams' condition through its effect on their feeding behavior, it is not likely to increase the risk of heavy metal toxicity to the population. However, PHC contamination of sediment could alter patterns of metal transfer in the marine benthic community and change the food web due to changes in feeding behavior.

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